Combination Therapy Using tPA and Edaravone Improves the Neurotoxic Effect of tPA

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Summary

In acute ischemic stroke patients, administration of tissue plasminogen activator (tPA) was proven to improve clinical outcome. On the other hand, neurotoxic effects of tPA have been reported in animal experimental studies. Using a rat thromboembolic stroke model, we examined whether or not the free radical scavenger, edaravone, could attenuate such neurotoxic effect of tPA administered for the purpose of fibrinolysis. Even when early recanalization was induced by administering tPA at 30 minutes after the onset of ischemia, significant amount of tPA was extravasated through the cerebral vessels. Edaravone significantly attenuated extravasation of tPA. Combination therapy using tPA and edaravone appears to be a promising strategy for diminishing the negative effects of tPA.

Introduction

Early restoration of cerebral blood flow by the administraion of tissue plasminogen activator (tPA) can improve the clinical outcome of acute ischemic stroke patients [1]. However, deleterious effects of tPA have also been reported in experimental studies [2]. Since tPA appears to promote neuroprotective as well as neurotoxic outcomes in ischemic stroke, future fibrinolytic therapy should aim to diminish the deleterious effects of tPA. In the present study, we examined whether or not the novel free radical scavenger, edaravone, might have a potential for inhibiting extravasation of tPA through the cerebral vessels.

Methods

Thromboembolic Stroke Model

Male Sprague-Dawley rats weighing 250 to 300 g were employed for the thromboembolic stroke model. Rats were anesthetized with 1-1.5% halothane in air-oxygen mixture under spontaneous respiration. Subsequent procedures for inducing thromboembolic stroke were performed according to the methods described by Kano et Al[3].

tPA Administration

tPA was given at 30 minutes (n=8) or two hours (n=8) after the induction of ischemia. tPA solution (Alteplase, 10 mg/kg, 1 mg/ 1 mL in saline) was administered using an infusion pump over a period of 30 minutes through the femoral vein.

Edaravone Administration

In edaravone treated animals, tPA and edaravone were given concomitantly. Edaravone solution (3 mg/kg, 3 mg/1 mL) was injected intravenously at 30 minutes (n=8) or two hours (n=8) after the induction of ischemia, and tPA solution was administered thereafter.

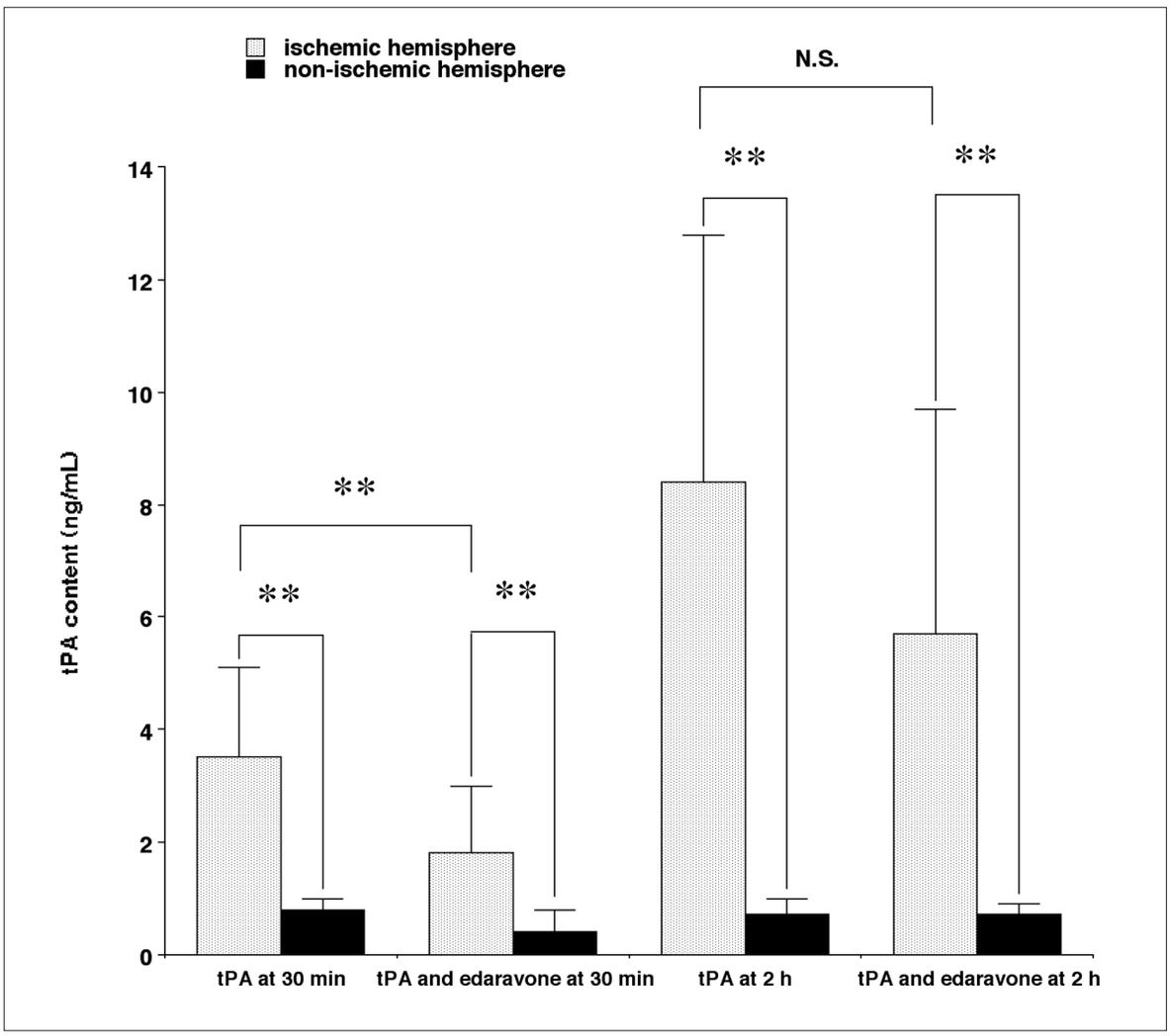

Figure 1 tPA content in the ischemic hemisphere and in the non-ischemic hemisphere of rats treated with tPA at 30 minutes after the onset of ischemia, rats treated with tPA and edaravone at 30 minutes, rats treated with tPA at 2 hours, and rats treated with tPA and edaravone at 2 hours. Values are expressed in ng/mL of homogenized brain in saline (means ± SD). **$p<0.01$.

Laser Doppler Flowmeter

To assess the induction of ischemia and restoration of perfusion, we evaluated the cerebral blood flow (CBF) by laser Doppler flowmetry (LDF, Omega Flow, Neuroscience Inc.) at the ischemic core of the model.

Quantification of tPA

Quantification of the extravasated tPA was carried out by enzyme-linked immunosorbent assay (ELISA) methods. At two hours after the beginning of edaravone or tPA administration, rats were sacrificed by injecting an overdose of pentobarbital sodium (100 mg/kg). The decapitated rat forebrain was cut into their two hemispheres, and each hemisphere was homogenized in a ninefold volume of added saline and centrifuged. The supernatant fluid was processed for analysis according to the methods of Ranby et Al[4].

An ANOVA followed by post hoc two-tailed t-tests with corrections for multiple groups was performed to compare the various outcomes among the different groups of animals. Differences with a value of $p<0.05$ were considered as statistically significant.

Results

LDF Measurement of CBF

Just after the injection of microclots, the CBF declined to levels below 20% of the preischemic baselines. At the time of tPA infusion, the CBF had increased slightly but remained below 30% of the baselines. At two hours after the onset of tPA administration, the CBF had recovered significantly to above 80% of the baselines.

Quantification of tPA

In the animals prepared for tPA administration at 30 minutes after the induction of ischemia, the tPA content in the ischemic hemisphere was significantly higher than that in the non-ischemic hemisphere: 3.5 ± 1.6 and 0.8 ± 0.2 ng/mL (mean $\pm$ SD) of the homogenized brain in saline, respectively ($p < 0.01$) (figure 1). By administering tPA with edaravone, the tPA content in the ischemic hemisphere was significantly decreased: 1.8 ± 1.2 ng/mL ($p < 0.01$) (figure 1). In the animals prepared for tPA administration at two hours after, the tPA content in the ischemic hemisphere was significantly higher than that in the non-ischemic hemisphere: 8.4 ± 4.4 and 0.7 ± 0.3 ng/mL, respectively ($p < 0.01$) (figure 1). By administering tPA with edaravone, the tPA content in the ischemic hemisphere was decreased but not significantly: 5.7 ± 4.0 ng/mL (figure 1). As each hemisphere was homogenized in a ninefold volume of added saline, the actual concentration of tPA in the brain was estimated to be 10 times higher than the above values.

Discussion

The present data demonstrate that significant amounts of tPA were extravasated through the cerebral vessels in association with ischemia/reperfusion, even when the tPA was administered at 30 minutes after the induction of ischemia, and the free radical scavenger, edaravone, significantly attenuated such extravasation of tPA. Much evidence has been accumulated to suggest that oxygen free radicals contribute to ischemia-reperfusion-induced brain injury. Edaravone traps a variety of free radical species including hydroxyl radicals[5]. In the present study, edaravone appeared to act directly on the reperfused cerebral vessels and to attenuate the vascular permeability to tPA.

In a previous in vitro study, tPA alone exhibited toxic effects on the viability of neurons only at very high concentrations of the order of μ g/mL[6]. In the present study, the tPA concentration in the ischemic hemisphere was of the order of ng/mL. However, such a concentration of tPA could be sufficiently high to amplify the neuronal damage when ischemic insult has already occurred. Further studies are needed to determine whether or not tPA concentration of the order of ng/mL do actually aggravate ischemic neuronal damage.

Conclusions

Edaravone is a unique anti-ischemic drug that can protect the cerebral vessels as well as neurons from ischemic injury. Based on the results of the present study, for acute ischemic stroke patients, combination therapy using tPA with edaravone appears to be a reasonable strategy for diminishing the negative effects of tPA.

References

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